FILE 'MEDLINE' ENTERED AT 16:19:35 ON 09 FEB 2000

FILE 'BIOSIS' ENTERED AT 16:19:35 ON 09 FEB 2000

=> s Yeast and (g-protein or GPCR or GPCRs) and (secret? or peptide library)

L1 42 YEAST AND (G-PROTEIN OR GPCR OR GPCRS) AND (SECRET? OR PEPTIDE LIBRARY)

=> d bib abs 1-42

L1 ANSWER 1 OF 42 MEDLINE
AN 1999365150 MEDLINE
DN 99365150
TI Multiple sex pheromones and receptors of a mushroom-producing fungus elicit mating in **yeast**.
AU Fowler T J; DeSimone S M; Mitton M F; Kurjan J; Raper C A
CS Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, Vermont 05405, USA.
SO MOLECULAR BIOLOGY OF THE CELL, (1999 Aug) 10 (8) 2559-72.
Journal code: BAU. ISSN: 1059-1524.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-AF148501; GENBANK-AF148500
EM 199912
EW 19991203
AB The mushroom-producing fungus Schizophyllum commune has thousands of mating types defined, in part, by numerous lipopeptide pheromones and their **G protein**-linked receptors. Compatible combinations of pheromones and receptors encoded by different mating types regulate a pathway of sexual development leading to mushroom formation and meiosis. A complex set of pheromone-receptor interactions maximizes the likelihood of outbreeding; for example, a single pheromone can activate more than one receptor and a single receptor can be activated by more than one pheromone. The current study demonstrates that the sex pheromones and receptors of Schizophyllum, when expressed in Saccharomyces cerevisiae, can substitute for endogenous pheromone and receptor and induce the **yeast** pheromone response pathway through the **yeast** **G protein**. **Secretion** of active Schizophyllum pheromone requires some, but not all, of the biosynthetic machinery used by the **yeast** lipopeptide pheromone a-factor. The specificity of interaction among pheromone-receptor pairs in Schizophyllum was reproduced in **yeast**, thus providing a powerful system for exploring molecular aspects of pheromone-receptor interactions for a class of seven-transmembrane-domain receptors common to a wide range of organisms.

L1 ANSWER 2 OF 42 MEDLINE
AN 1999278370 MEDLINE
DN 99278370
TI Identification of amino acid residues critical for aggregation of human
CC

chemokines macroph inflammatory protein (MIP)-1al[redacted], MIP-1beta, and RANTES. Characteri on of active disaggregated che[redacted]ine variants.

AU Czaplewski L G; McKeating J; Craven C J; Higgins L D; Appay V; Brown A; Dudgeon T; Howard L A; Meyers T; Owen J; Palan S R; Tan P; Wilson G; Woods N R; Heyworth C M; Lord B I; Brotherton D; Christison R; Craig S; Cribbes S; Edwards R M; Evans S J; Gilbert R; Morgan P; Hunter M G; et al

CS British Biotech Pharmaceuticals Ltd., Watlington Road, Oxford OX4 5LY, United Kingdom.. czaplewski@britbio.co.uk

SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1999 Jun 4) 274 (23) 16077-84. Journal code: HIV. ISSN: 0021-9258.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals; Cancer Journals

OS PDB-1B50; PDB-1B53

EM 199909

EW 19990901

AB Human CC chemokines macrophage inflammatory protein (MIP)-1alpha, MIP-1beta, and RANTES (regulated on activation normal T cell expressed) self-associate to form high-molecular mass aggregates. To explore the biological significance of chemokine aggregation, nonaggregating variants were sought. The phenotypes of 105 hMIP-1alpha variants generated by systematic mutagenesis and expression in **yeast** were determined. hMIP-1alpha residues Asp26 and Glu66 were critical to the self-association process. Substitution at either residue resulted in the formation of essentially homogenous tetramers at 0.5 mg/ml. Substitution of identical or analogous residues in homologous positions in both hMIP-1beta and RANTES demonstrated that they were also critical to aggregation. Our analysis suggests that a single charged residue at either position 26 or 66 is insufficient to support extensive aggregation and that two charged residues must be present. Solution of the three-dimensional NMR structure of hMIP-1alpha has enabled comparison of these residues in hMIP-1beta and RANTES. Aggregated and disaggregated forms of hMIP-1alpha, hMIP-1beta, and RANTES generally have equivalent **G-protein**-coupled receptor-mediated biological potencies. We have therefore generated novel reagents to evaluate the role of hMIP-1alpha, hMIP-1beta, and RANTES aggregation in vitro and in vivo. The disaggregated chemokines retained their human immunodeficiency virus (HIV) inhibitory activities. Surprisingly, high concentrations of RANTES, but not disaggregated RANTES variants, enhanced infection of cells by both M- and T-tropic HIV isolates/strains. This observation has important implications for potential therapeutic uses of chemokines implying that disaggregated forms may be necessary for safe clinical investigation.

L1 ANSWER 3 OF 42 MEDLINE

AN 1998371016 MEDLINE

DN 98371016

TI A 29-kilodalton Golgi soluble N-ethylmaleimide-sensitive factor attachment protein receptor (Vti1-rp2) implicated in protein trafficking in the **secretory** pathway.

AU Xu Y; Wong S H; Tang B L; Subramaniam V N; Zhang T; Hong W

CS Membrane Biology Laboratory, Institute of Molecular and Cell Biology, Singapore 117609, Singapore.

SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1998 Aug 21) 273 (34) 21783-9. Journal code: HIV. ISSN: 0021-9258.

CY United States

DT Journal; Article; (JOURNAL ARTICLE)

LA English

FS Priority Journals; Cancer Journals

OS GENBANK-AF035823

EM 199811

EW 19981103
AB Expressed sequence [redacted]s coding for a potential SNARE [redacted]oluble N-ethylmaleimide-sensitive factor attachment protein receptor) were revealed during data base searches. The deduced amino acid sequence of the complete coding region predicts a 217-residue protein with a COOH-terminal hydrophobic membrane anchor. Affinity-purified antibodies raised against the cytoplasmic region of this protein specifically detect a 29-kilodalton integral membrane protein enriched in the Golgi membrane. Indirect immunofluorescence microscopy reveals that this protein is mainly associated with the Golgi apparatus. When detergent extracts of the Golgi membrane are incubated with immobilized glutathione S-transferase alpha soluble N-ethylmaleimide-sensitive factor attachment protein (GST-alpha-SNAP), this protein was specifically retained. This protein has been independently identified and termed Vti1-rp2, and it is homologous to Vti1p, a **yeast** Golgi SNARE. We further show that Vti1-rp2 can be qualitatively coimmunoprecipitated with Golgi syntaxin 5 and syntaxin 6, suggesting that Vti1-rp2 exists in at least two distinct Golgi SNARE complexes. In cells microinjected with antibodies against Vti1-rp2, transport of the envelope protein (**G-protein**) of vesicular stomatitis virus from the endoplasmic reticulum to the plasma membrane was specifically arrested at the Golgi apparatus, providing further evidence for functional importance of Vti1-rp2 in protein trafficking in the **secretory** pathway.

L1 ANSWER 4 OF 42 MEDLINE
AN 1998169075 MEDLINE
DN 98169075
TI Structure of the Sec7 domain of the Arf exchange factor ARNO.
AU Cherfils J; Menetrey J; Mathieu M; Le Bras G; Robineau S; Beraud-Dufour S; Antonny B; Chardin P
CS Laboratoire d'Enzymologie et Biochimie Structurales, CNRS UPR 9063, Gif-sur-Yvette, France.. cherfils@lebs.cnrs-gif.fr
SO NATURE, (1998 Mar 5) 392 (6671) 101-5.
Journal code: NSC. ISSN: 0028-0836.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
OS PDB-1PBV
EM 199805
EW 19980504
AB Small G proteins switch from a resting, GDP-bound state to an active, GTP-bound state. As spontaneous GDP release is slow, guanine-nucleotide-exchange factors (GEFs) are required to promote fast activation of small G proteins through replacement of GDP with GTP in vivo. Families of GEFs with no sequence similarity to other GEF families have now been assigned to most families of small G proteins. In the case of the small **G protein** Arf1, the exchange of bound GDP for GTP promotes the coating of **secretory** vesicles in Golgi traffic. An exchange factor for human Arf1, ARNO, and two closely related proteins, named cytohesin 1 and GPS1, have been identified. These three proteins are modular proteins with an amino-terminal coiled-coil, a central Sec7-like domain and a carboxy-terminal pleckstrin homology domain. The Sec7 domain contains the exchange-factor activity. It was first found in Sec7, a **yeast** protein involved in **secretion**, and is present in several other proteins, including the **yeast** exchange factors for Arf, Geal and Gea2. Here we report the crystal structure of the Sec7 domain of human ARNO at 2 A resolution and the identification of the site of interaction of ARNO with Arf.

L1 ANSWER 5 OF 42 MEDLINE
AN 1998036061 MEDLINE
DN 98036061
TI A genetic selection for isolating cDNAs encoding **secreted** proteins.
AU Jacobs K A; Collins-Racie L A; Colbert M; Duckett M; Golden-Fleet M; Kelleher K; Kriz R; LaVallie E R; Merberg D; Spaulding V; Stover J; Williamson M J; McCoy J M
CS Genetics Institute, Inc., Cambridge, MA 02140, USA.. kjacobs@genetics.com
SO GENE, (1997 Oct 1) 198 (1-2) 289-96.
Journal code: FOP. ISSN: 0378-1119.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
OS GENBANK-AF002985; GENBANK-AF002986
EM 199802
AB We describe a simple, rapid technique for simultaneously isolating large numbers of cDNAs encoding **secreted** proteins. The technique makes use of a facile genetic selection performed in a strain of Saccharomyces cerevisiae deleted for its endogenous invertase gene. A cDNA cloning vector which carries a modified invertase gene lacking its leader sequence is used in conjunction with this strain. Heterologous **secreted** genes fused appropriately upstream of this defective invertase provide the necessary signals to restore **secretion**, allowing the **yeast** to grow on sugars such as sucrose or raffinose. This microbial growth selection facilitates scanning cDNA libraries containing millions of clones, enabling the wholesale identification of novel **secreted** proteins without the need for specific bioassays. The technique is similar to one previously described (Klein et al. (1996) Proc. Natl. Acad. Sci. USA 93, 7108-7113). We describe results using a cDNA library derived from activated human peripheral blood mononuclear cells (PBMC). Genes identified from this library encoded signal sequences of proteins of diverse structure, function, and cellular location such as cytokines, type 1 and type 2 transmembrane proteins, and proteins found in intracellular organelles. In addition, a number of novel **secreted** proteins were identified, including a chemokine and a novel **G-protein**-coupled receptor. Since signal sequences possess features conserved throughout evolution, the procedure can be used to isolate genes encoding **secreted** proteins from both eukaryotes and prokaryotes.

L1 ANSWER 6 OF 42 MEDLINE
AN 97334092 MEDLINE
DN 97334092
TI Phosphatidic acid formation by phospholipase D is required for transport from the endoplasmic reticulum to the Golgi complex.
AU Bi K; Roth M G; Ktistakis N T
CS Department of Biochemistry, University of Texas, Southwestern Medical Center, Dallas, Texas 75235-9038, USA.
SO CURRENT BIOLOGY, (1997 May 1) 7 (5) 301-7.
Journal code: B44. ISSN: 0960-9822.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199708
EW 19970804
AB BACKGROUND: Lipid molecules may play a regulatory role in the **secretory** pathway of mammals and **yeast**. The lipid hydrolase phospholipase D (PLD) is one candidate for mediating regulation of **secretion**, based on the location of this enzyme and its

requirements for activation. RESULTS: We found that primary alcohols, which block formation of phosphatidic acid (PA) by PLD, inhibited the transport of two different viral glycoproteins from the endoplasmic reticulum to the Golgi complex in Chinese hamster ovary cells. Corresponding secondary alcohols, which are much less potent in blocking PA formation, were also less effective in blocking transport of the glycoproteins. The block in glycoprotein transport imposed by primary alcohols was reversed when PA, in the form of liposomes, was exogenously supplied to the culture medium. CONCLUSIONS: We suggest that the earliest site of regulation of membrane transport by PLD is within the intermediate compartment between the endoplasmic reticulum and the Golgi complex.

L1 ANSWER 7 OF 42 MEDLINE
AN 97322802 MEDLINE
DN 97322802
TI Functional expression of bovine opsin in the methylotrophic **yeast** Pichia pastoris.
AU Abdulaev N G; Popp M P; Smith W C; Ridge K D
CS Center for Advanced Research in Biotechnology, National Institute of Standards and Technology, Rockville, Maryland, USA.
NC EY11112 (NEI)
EY06641 (NEI)
SO PROTEIN EXPRESSION AND PURIFICATION, (1997 Jun) 10 (1) 61-9.
Journal code: BJV. ISSN: 1046-5928.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199709
EW 19970903
AB The methylotrophic **yeast** Pichia pastoris was examined for functional expression of bovine opsin. An expression plasmid was constructed where the bovine opsin gene was placed downstream from the P. pastoris alcohol oxidase 1 gene promoter and fused at its amino-terminus to the acid phosphatase **secretion** signal. Quantitative-competitive PCR analysis of a stable **yeast** transformant showed that one copy of the opsin gene was integrated into the **yeast** genome. The expression level in this transformant corresponded to approximately 0.3 mg of opsin per liter of cell culture (A600 = 1.0). Sucrose density sedimentation analysis indicated that the opsin was associated exclusively with the membrane fraction. Similar to retinal opsin, P. pastoris-expressed opsin migrated as a single band of approximately 37 kDa on SDS-PAGE and showed high mannose N-glycosylation. A portion of the expressed opsin (approximately 4-15%) reacted with 11-cis-retinal to form the rhodopsin chromophore (lambda max 500 nm), and after purification showed ground and excited state spectral characteristics indistinguishable from those of the native pigment. Further, the metarhodopsin-II-mediated **G-protein** -activating potential of **yeast** expressed rhodopsin was similar to that of native rhodopsin. These results show that P. pastoris cells have the capacity to functionally express bovine opsin.

L1 ANSWER 8 OF 42 MEDLINE
AN 97233199 MEDLINE
DN 97233199
TI Rab3A small GTP-binding protein in Ca(2+)-dependent exocytosis.
AU Takai Y; Sasaki T; Shirataki H; Nakanishi H
CS Department of Molecular Biology and Biochemistry, Osaka University Medical School, Suita, Japan.
SO GENES TO CELLS, (1996 Jul) 1 (7) 615-32. Ref: 108
Journal code: CUF. ISSN: 1356-9597.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)

(REVIEW, ACADEMIC)
LA English
FS Priority Journals
EM 199707
EW 19970701
AB There exists a small GTP-binding protein (**G protein**) superfamily, consisting of more than 50 members, from **yeast** to mammal. The Rab family belongs to this superfamily and is implicated in intracellular vesicle trafficking. Rab3A small **G protein** is a member of the Rab3 subfamily which belongs to this Rab family. The regulators and downstream targets of Rab3A have been isolated, and evidence is accumulating that Rab3A and these Rab3A-interacting proteins are involved in Ca(2+)-dependent exocytosis, particularly in neurotransmitter release from nerve terminals.

L1 ANSWER 9 OF 42 MEDLINE
AN 97181336 MEDLINE
DN 97181336
TI Investigation of growth hormone releasing hormone receptor structure and activity using **yeast** expression technologies.
AU Kajkowski E M; Price L A; Pausch M H; Young K H; Ozenberger B A
CS American Cyanamid Company, Princeton, New Jersey 08543, USA.
SO JOURNAL OF RECEPTOR AND SIGNAL TRANSDUCTION RESEARCH, (1997 Jan-May) 17 (1-3) 293-303.
Journal code: CCU. ISSN: 1079-9893.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199707
EW 19970702
AB Growth hormone releasing hormone (GHRH) is the positive regulator of growth hormone synthesis and **secretion** in the anterior pituitary. The peptide confers activity by binding to a seven transmembrane domain **G protein**-coupled receptor. Signal transduction proceeds through subsequent G alpha s stimulation of adenylyl cyclase. To investigate ligand/receptor and receptor/**G protein** associations, the human GHRH receptor was expressed in a modified S. cerevisiae strain which allows for facile measurement of receptor activity by cell prototrophy mediated by a reporter gene coupled to the **yeast** pheromone response pathway. GHRH-dependent signal activation in this system required the substitution of **yeast** G alpha protein with proteins containing C-terminal regions of G alpha s. A D60G variant (analogous to the little mouse mutation) of the receptor failed to respond to agonist. In parallel studies, GHRH29 and the N-terminal extracellular region of the receptor were expressed as Gal4 fusion proteins in a 2-hybrid assay. A specific interaction between these proteins was readily observed. The D60G mutation was engineered into the receptor fusion protein. This protein failed to interact with the ligand fusion, confirming the specificity of the association between unmodified proteins. These two **yeast** expression technologies should prove invaluable in additional structure/activity analyses of this ligand/receptor pair as well as other peptide ligands and receptors.

L1 ANSWER 10 OF 42 MEDLINE
AN 97100951 MEDLINE
DN 97100951
TI A human exchange factor for ARF contains Sec7- and pleckstrin-homology domains.
AU Chardin P; Paris S; Antonny B; Robineau S; Beraud-Dufour S; Jackson C L; Chabre M
CS Institut de Pharmacologie Moleculaire et Cellulaire du CNRS, Valbonne,France.. chardin@unice.fr
SO NATURE, (1996 Dec 5) 384 (6608) 481-4.
Journal code: NSC. ISSN: 0028-0836.
CY ENGLAND: United Kingdom

DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
OS GENBANK-X99753
EM 199703
AB The small **G protein** ARF1 is involved in the coating of vesicles that bud from the Golgi compartments. Its activation is controlled by as-yet unidentified guanine-nucleotide exchange factors. Gea1, the first ARF exchange factor to be discovered in **yeast**, is a large protein containing a domain of homology with Sec7, another **yeast** protein that is also involved in **secretion**. Here we characterized a smaller human protein (relative molecular mass 47K) named ARNO, which contains a central Sec7 domain that promotes guanine-nucleotide exchange on ARF1. ARNO also contains an amino-terminal coiled-coil motif and a carboxy-terminal pleckstrin-homology (PH) domain. The PH domain mediates an enhancement of ARNO exchange activity by negatively charged phospholipid vesicles supplemented with phosphatidylinositol bisphosphate. The exchange activity of ARNO is not inhibited by brefeldin A, an agent known to block vesicular transport and inhibit the exchange activity on ARF1 in cell extracts. This suggests that a regulatory component which is sensitive to brefeldin A associates with ARNO in vivo, possibly through the amino-terminal coiled-coil. We propose that other proteins with a Sec7 domain regulate different members of the ARF family.

L1 ANSWER 11 OF 42 MEDLINE
AN 96394622 MEDLINE
DN 96394622
TI The role of charged residues in determining transmembrane protein insertion orientation in **yeast**.
AU Harley C A; Tipper D J
CS Department of Molecular Genetics and Microbiology, University of Massachusetts Medical School, Worcester, Massachusetts 01655, USA.
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Oct 4) 271 (40) 24625-33. Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199701
EW 19970104
AB The first 79 residues of the **yeast** Ste2p **G protein**-coupled pheromone receptor, including the negatively charged N-terminal domain, the first transmembrane segment, and the following positively charged cytoplasmic loop, has been fused to a Kex2p-cleavable beta-lactamase reporter. Insertion orientation was determined by analysis of cell-associated and **secreted** beta-lactamase activities and independently corroborated by analysis of membrane association and glycosylation patterns. This fusion inserts with exclusively N terminus exofacial (Nexo) topology, serving as a model type III membrane protein. Orientation is unaffected by removal of all three positively charged residues in the cytoplasmic loop or by deletion of all but 12 residues from the N-terminal domain. The residual -2 N-terminal charge apparently provides a signal sufficient to determine Nexo topology. This is entirely consistent with the statistically derived rule in which the charge difference, Delta(C-N), counted for the 15 immediately flanking residues, is the primary topology determinant. Mutations altering Delta(C-N) to zero favors Nexo insertion by 3 to 1, whereas increasingly negative values cause increasing inversion of orientation. All results are consistent with the charge difference rule and indicate that whereas positive charges promote cytoplasmic retention, negative charges promote translocation.

L1 ANSWER 12 OF 42 MEDLINE
AN 96355350 MEDLINE
DN 96355350
TI Regulation of membrane and subunit interactions by N-myristoylation of a **G protein** alpha subunit in **yeast**.
AU Song J; Hirschman J; Gunn K; Dohlman H G
CS Department of Pharmacology, Boyer Center for Molecular Medicine, Yale University School of Medicine, New Haven, Connecticut 06536-0812, USA.
NC GM34719 (NIGMS)
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1996 Aug 23) 271 (34) 20273-83. Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199612
AB Initiation of the mating process in **yeast** Saccharomyces cerevisiae requires the action of **secreted** pheromones and **G protein**-coupled receptors. As in other eukaryotes, the **yeast G protein** alpha subunit undergoes N-myristoylation (GPA1 gene product, Gpa1p). This modification appears to be essential for function, since a myristoylation site mutation exhibits the null phenotype in vivo (gpa1(G2A)). Here we examine how myristoylation affects Gpa1p activity in vitro. We show that the G2A mutant of Gpa1p, when fused with glutathione S-transferase, can still form a complex with the **G protein** betagamma subunits. The complex is stabilized by GDP and is dissociated upon treatment with guanosine 5'-O-(thiotriphosphate). In addition, there is no apparent difference in the relative binding affinity of Gbetagamma for mutant and wild-type Gpa1p. Using sucrose density gradient fractionation of cell membranes, Gpa1p associates normally with the plasma membrane whereas Gpa1pG2A is mislocalized to a microsomal membrane fraction. A portion of Gbetagamma is also mislocalized in these cells, as it is in a gpa1Delta strain. In contrast, wild-type Gpa1p reaches the plasma membrane in cells that do not express Gbetagamma or cell surface receptors. These findings indicate that mislocalization of Gpa1pG2A is not caused by a redistribution of Gbetagamma, nor is it the result of any difference in Gbetagamma binding affinity. These data suggest that myristoylation is required for specific targeting of Gpa1p to the plasma membrane, where it is needed to interact with the receptor and to regulate the release of Gbetagamma.

L1 ANSWER 13 OF 42 MEDLINE
AN 96347538 MEDLINE
DN 96347538
TI **Yeast** alpha mating factor structure-activity relationship derived from genetically selected peptide agonists and antagonists of Ste2p.
AU Manfredi J P; Klein C; Herrero J J; Byrd D R; Trueheart J; Wiesler W T; Fowlkes D M; Broach J R
CS Cadus Pharmaceutical Corporation, Tarrytown, New York 10591-6705, USA.
SO MOLECULAR AND CELLULAR BIOLOGY, (1996 Sep) 16 (9) 4700-9. Journal code: NGY. ISSN: 0270-7306.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199611
AB alpha-Factor, a 13-amino-acid pheromone **secreted** by haploid alpha cells of Saccharomyces cerevisiae, binds to Ste2p, a seven-transmembrane, **G-protein**-coupled receptor present on haploid alpha cells, to activate a signal transduction pathway

required for conjug[redacted]ion and mating. To determine the structural requirements for al[redacted]-factor activity, we developed [redacted]genetic screen to identify from random and semirandom libraries novel peptides that function as agonists or antagonists of Ste2p. The selection scheme was based on autocrine strains constructed to **secrete** random peptides and respond by growth to those that were either agonists or antagonists of Ste2p. Analysis of a number of peptides obtained by this selection procedure indicates that Trp1, Trp3, Pro8, and Gly9 are important for agonist activity specifically. His2, Leu4, Leu6, Pro10, a hydrophobic residue 12, and an aromatic residue 13 are important for both agonist and antagonist activity. Our results also show that activation of Ste2p can be achieved with novel, unanticipated combinations of amino acids. Finally, the results suggest the utility of this selection scheme for identifying novel ligands for mammalian **G-protein**-coupled receptors heterologously expressed in S. cerevisiae.

L1 ANSWER 14 OF 42 MEDLINE
AN 96226025 MEDLINE
DN 96226025
TI Immunodominant epitopes defined by a **yeast**-expressed library of random fragments of the rabies virus glycoprotein map outside major antigenic sites.
AU Lafay F; Benmansour A; Chebli K; Flamand A
CS Laboratoire de genetique des virus, CNRS, Gif-sur-Yvette, France.
SO JOURNAL OF GENERAL VIROLOGY, (1996 Feb) 77 (Pt 2) 339-46.
Journal code: I9B. ISSN: 0022-1317.
CY ENGLAND: United Kingdom
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199608
AB Nineteen **yeast** colonies **secreting** rabies virus glycoprotein (G) peptides immunoreactive with polyclonal anti-rabies virus sera were selected from a random expression library. The peptides, around 80 amino acids long, spanned amino acids 54-494 of the **G protein**. These peptides, together with two constructions including, respectively, immunodominant sites II and III, were analysed for their immunoreactivity with 40 anti-**G protein** monoclonal antibodies (MAbs) composed of 12 MAbs that reacted with SDS-treated protein in Western blot under reducing conditions (WB+) and 28 representative MAbs that did not react after denaturation (WB-). This last category represents 98% of anti-rabies virus G MAbs. None of the WB- MAbs bound peptides. Of the 12 WB+ MAbs, one bound two peptides situated before the transmembrane domain of the protein and six bound peptides overlapping a region situated between amino acids 223 and 276. These six MAbs define a new antigenic region that would be considered 'immunodominant' if the peptide strategy had been used to study the antigenicity of the protein; however, this region is only recognized by about 1% of our MAbs. Three of these WB+ MAbs had significant neutralizing activity; two were used for the selection of antigenic mutants (MAR mutants). Some mutants had a substitution within the region delimited by the peptides, confirming the pertinence of both the peptide and escape mutant approaches. However, a few mutants had a substitution outside the peptide-delimited region, suggesting that remote mutation(s) could affect epitope accessibility in the native protein.

L1 ANSWER 15 OF 42 MEDLINE
AN 96198506 MEDLINE

DN 96198506
TI Mitochondrial FAD-g[redacted]erophosphate dehydrogenase and [redacted] **protein**-coupled inwardly rectifying K+ channel: No evidence for linkage in maturity-onset diabetes of the young or NIDDM [published erratum appears in Diabetes 1996 Sep;45(9):1285].
AU Warren-Perry M G; Stoffel M; Saker P J; Zhang Y; Brown L J; MacDonald M J;
Turner R C
CS Diabetes Research Laboratories, Radcliffe Infirmary, Oxford, U.K.
SO DIABETES, (1996 May) 45 (5) 639-41.
Journal code: E8X. ISSN: 0012-1797.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
EM 199608
AB Two genes that have potentially important regulatory roles in insulin **secretion** are both located on chromosome 2q24.1. **G-protein**-coupled muscarinic potassium channel (GIRK1) is an inwardly rectifying K+ channel that helps to maintain the resting potential and excitability of cells. Mitochondrial FAD-linked glycerophosphate dehydrogenase (m-GDH) catalyzes a rate-limiting step of the glycerol phosphate shuttle in pancreatic islets. Reduced m-GDH activity has been demonstrated in islets isolated from diabetic subjects compared with islets from nondiabetic control subjects and from the diabetic GK rat. To study the relationship between these candidate genes and NIDDM, we have examined a simple tandem-repeat polymorphism (STRP) close to both the KCN J3 (GIRK1) locus and the m-GDH locus. In a linkage study of three maturity-onset diabetes of the young (MODY) pedigrees, not linked to MODY1, MODY2, or MODY3, a cumulative score of - 9.6 at a recombination fraction of theta = 0 excluded linkage. In a population-association study, no linkage disequilibrium for the STRP was found between 190 unselected NIDDM patients and 60 geographically and age-matched white nondiabetic subjects (chi2 = 1.51 on 3 df, P = 0.68). Thus, mutations involving the genes for GIRK1 or FAD-glycerophosphate dehydrogenase are unlikely to cause MODY, and a common mutation in either gene is unlikely to contribute to NIDDM in whites. These data do not exclude mutations in some families or other ethnic groups.

L1 ANSWER 16 OF 42 MEDLINE
AN 96026433 MEDLINE
DN 96026433
TI Interaction of the protein nucleobindin with G alpha i2, as revealed by the **yeast** two-hybrid system.
AU Mochizuki N; Hibi M; Kanai Y; Insel P A
CS Department of Pharmacology, University of California San Diego, La Jolla 92093-0636, USA.
NC GM31987 (NIGMS)
GM40781 (NIGMS)
HL35018 (NHLBI)
+
SO FEBS LETTERS, (1995 Oct 9) 373 (2) 155-8.
Journal code: EUH. ISSN: 0014-5793.
CY Netherlands
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
OS GENBANK
EM 199602
AB The heterotrimeric **G protein**, G alpha i2, transduces signals from seven membrane spanning receptors to effectors such as adenylyl cyclase and ion channels. The purpose of this study was to identify these or other cellular proteins that interact with G alpha i2 by use of the **yeast** two-hybrid system. A human B cell cDNA library was screened by this system using full length G alpha i2. Four positive

colonies were obtained. Two of the four were identified as nucleobindin, a calcium binding protein and a putative antigen to which anti-nuclear antibodies are generated in mice with a disorder that resembles systemic lupus erythematosus. Nucleobindin has a leucine zipper, EF hands, and a signal peptide sequence and is thought to localize to the nucleus as well as being **secreted**. The specificity of intehraction between G alpha i2 and nucleobindin was confirmed by an in vitro binding assay using recombinant proteins. Transfection of G alpha i2 and nucleobindin in COS cells increased G alpha i2 expression relative to cells transfected with G alpha i2 and mock vector. Our results indicate that the **yeast** two-hybrid system provides a means to identify novel proteins that interact with G alpha proteins. Nucleobindin appears to represent one of those proteins.

L1 ANSWER 17 OF 42 MEDLINE
AN 95246962 MEDLINE
DN 95246962
TI Isolation of a cDNA clone encoding a KATP channel-like protein expressed in insulin-**secreting** cells, localization of the human gene to chromosome band 21q22.1, and linkage studies with NIDDM.
AU Tsaur M L; Menzel S; Lai F P; Espinosa R 3rd; Concannon P; Spielman R S; Hanis C L; Cox N J; Le Beau M M; German M S; et al
CS Howard Hughes Medical Institute, University of California, San Francisco, USA.
NC DK-20595 (NIDDK)
DK-47486 (NIDDK)
DK-47487 (NIDDK)
+
SO DIABETES, (1995 May) 44 (5) 592-6.
Journal code: E8X. ISSN: 0012-1797.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Abridged Index Medicus Journals; Priority Journals
OS GENBANK-U21937
EM 199508
AB The metabolism of glucose in insulin-**secreting** cells leads to closure of ATP-sensitive K+ channels (KATP), an event that initiates the insulin **secretory** process. Defects in insulin **secretion** are a common feature of non-insulin-dependent diabetes mellitus (NIDDM), and the beta-cell KATP that couples metabolism and membrane potential is a candidate for contributing to the development of this clinically and genetically heterogeneous disorder. We screened a hamster insulinoma cDNA library by low-stringency hybridization with a probe coding for the **G-protein**-coupled inwardly rectifying K+ channel GIRK1/KGA and isolated clones encoding a protein, KATP-2, whose sequence is 90% similar to that of the recently described KATP-1, an ATP-sensitive K+ channel expressed in heart and other tissues. RNA blotting showed that KATP mRNA was present in insulin-**secreting** cells and brain but not in heart. To assess the contribution of KATP-2 to the development of NIDDM, the human KATP-2 gene (symbol KCNJ7) was isolated and mapped to chromosome band 21q22.1 by fluorescence in situ hybridization. A simple tandem repeat DNA polymorphism, D21S1255, was identified in the region of the KATP-2 gene, and linkage studies between this marker and NIDDM were carried out in a group of Mexican-American sib pairs with NIDDM. There was no evidence for linkage between D21S1255 and NIDDM, indicating that KATP-2 is not a major susceptibility gene in this population.

L1 ANSWER 18 OF 42 MEDLINE
AN 95050768 MEDLINE

DN 95050768
TI Syntaxin 5 regulates endoplasmic reticulum to Golgi transport.
AU Dascher C; Matteson J; Balch W E
CS Department of Cell Biology, Scripps Research Institute, La Jolla, California 92037.
NC GM 42336 (NIGMS)
RR07273 (NCRR)
RR08176
SO JOURNAL OF BIOLOGICAL CHEMISTRY, (1994 Nov 25) 269 (47) 29363-6.
Journal code: HIV. ISSN: 0021-9258.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199502
AB Syntaxins are a family of vesicular transport receptors that are involved in membrane traffic through both the constitutive and regulated **secretory** pathways. Syntaxins 1A/B,2,3, and 4 are principally associated with the plasma membrane. Two of the syntaxins, 1A and 1B, have been suggested to be the docking receptors for synaptic vesicles with the presynaptic membrane. The most distant member of the family, syntaxin 5, has been found in the Golgi region and has significant homology (35% identity) with Sed5p, an essential protein in **yeast** which is required for vesicular transport from the endoplasmic reticulum (ER) to the Golgi stack. Here we present evidence that syntaxin 5 performs an analogous function in ER to Golgi transport in mammalian cells. Transient expression of an hemagglutinin-tagged full-length clone of syntaxin 5 and a truncated mutant lacking the transmembrane domain inhibited the transport of vesicular stomatitis virus glycoprotein to the Golgi stack. Under these conditions, vesicular stomatitis virus glycoprotein accumulated in pre-Golgi intermediates, which were strongly enriched in syntaxin 5. Our results suggest that syntaxin 5 is the functional mammalian homologue of Sed5p and provides evidence for its role in regulating the potential targeting and/or fusion of carrier vesicles following export from the ER.

L1 ANSWER 19 OF 42 MEDLINE
AN 94373866 MEDLINE
DN 94373866
TI **Yeast** ts **secretory** mutation rgs1 is suppressed by the SEC4 gene of Saccharomyces cerevisiae.
AU Gerassimenko O G
CS Department of Molecular Biology, Faculty of Biology, Moscow State University, Russia.
SO CURRENT GENETICS, (1994 Feb) 25 (2) 178-9.
Journal code: CUG. ISSN: 0172-8083.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 199412
AB **Yeast** rgs1 cells accumulate **secretory** vesicles in the cytoplasm and stop the **secretion** of proteins at the restrictive temperature. The ts mutation rgs1 may be suppressed by several different genes; the S. cerevisiae SEC4 gene, encoding the small **G-protein** involved in the late **secretory** stage, is one of them. Synthetic lethality of the double rgs1 sec4 mutant is demonstrated.

L1 ANSWER 20 OF 42 MEDLINE
AN 94240188 MEDLINE
DN 94240188
TI **Yeast** as a model system for mammalian seven-transmembrane segment receptors.
AU Jeansonne N E
CS East Carolina University Medical School, Department of Pharmacology,

Greenville, North Carolina 27858.
SO PROCEEDINGS OF THE SOCIETY FOR EXPERIMENTAL BIOLOGY MEDICINE, (1994 May) 206 (1) 35-44. Ref: 115
Journal code: PXZ. ISSN: 0037-9727.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
General Review; (REVIEW)
(REVIEW, TUTORIAL)
LA English
FS Priority Journals; Cancer Journals
EM 199408
AB Investigators have used the budding **yeast** Saccharomyces cerevisiae as a model system in which to study the beta-adrenergic receptor, the T-cell receptor pathway, initiation of mammalian DNA replication, initiation of mammalian transcription, **secretion**, the CDC2 kinase system, cell cycle control, and aging, as well as the function of oncogenes. This list continues to grow with the discovery of an immunoglobulin heavy-chain binding homologue in **yeast**, an Rb binding protein homologue, and a possible **yeast** arrestin. **Yeast** is relatively easy to maintain, to grow, and to genetically manipulate. A single gene can be overexpressed, selectively mutated or deleted from its chromosomal location. In this way, the in vivo function of a gene can be studied. It has become reasonable to consider **yeast** as a model system for studying the seven transmembrane segments (7-TMS) receptor family. Currently, subtypes of the beta-adrenergic receptor are being studied in **yeast**. The receptor and its G alpha-**G-protein**, trigger the mating pheromone receptor pathway. This provides a powerful assay for determining receptor function. Studies expressing the muscarinic cholinergic receptor in **yeast** are underway. The **yeast** pheromone receptor belongs to this receptor family, sharing sequence and secondary structure homology. An effective strategy has been to identify a **yeast** pathway or process which is homologous to a mammalian system. The pathway is delineated in **yeast**, identifying other genetic components. Then **yeast** genes are used to screen for human homologues of these components. The putative human homologues are then expressed in **yeast** and in mammalian cells to determine function. When this type of "mixing and matching" works, **yeast** genetics can be a powerful tool.

L1 ANSWER 21 OF 42 MEDLINE
AN 92171926 MEDLINE
DN 92171926
TI Functional expression of rat M5 muscarinic acetylcholine receptor in **yeast**.
AU Huang H J; Liao C F; Yang B C; Kuo T T
CS Graduate Institute of Botany, National Taiwan University, Taipei, Republic of China.
SO BIOCHEMICAL AND BIOPHYSICAL RESEARCH COMMUNICATIONS, (1992 Feb 14) 182 (3) 1180-6.
Journal code: 9Y8. ISSN: 0006-291X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 199206
AB We have produced the rat M5 muscarinic acetylcholine receptor, an integral membrane protein, in the **yeast** Saccharomyces cerevisiae. This was achieved by placing an M5 gene in the **yeast** vector under the control of the **yeast** alpha-factor promoter and leader sequence. Northern blotting revealed the presence of M5 transcripts in **yeast** transformed with the M5 plasmid constructs. Crude extract prepared from

the transformant yeasts showed saturable binding of the muscarinic antagonist [3H]-N-methyl scopolamine ([3H]NMS) with Kd of 22.77 nM and Bmax of 134.76 fmole per mg protein. Results deduced from saturation binding assay of intact cell demonstrated clearly that the M5 receptor was translocated across the membrane of the endoplasmic reticulum using the **secretion** signal on alpha-leader sequence and its binding site was still functional. **Yeast** expressing M5 receptor did not exhibit cell-cycle arrest in the presence of carbachol, a acetylcholine agonist, indicating that the recombinant M5 receptor could not couple directly to the endogenous **yeast** pheromone signaling **G-protein**.

L1 ANSWER 22 OF 42 MEDLINE
AN 89384206 MEDLINE
DN 89384206
TI Reconstitution of transport from the ER to the Golgi complex in **yeast** using microsomes and permeabilized **yeast** cells.
AU Ruohola H; Kabcenell A K; Ferro-Novick S
CS Department of Cell Biology, Yale University School of Medicine, New Haven, Connecticut 06510.
NC GM 35421 (NIGMS)
SO METHODS IN CELL BIOLOGY, (1989) 31 143-54.
Journal code: MV4. ISSN: 0091-679X.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals
EM 198912
AB We have developed a highly efficient in vitro-transport assay that couples translocation across the ER membrane and transport to the Golgi complex using the **secreted** pheromone alpha-factor as a marker protein. Radiolabeled prepro-alpha-factor of high specific radioactivity is obtained by in vitro-translating this protein in a **yeast** lysate. Prepro-alpha-factor synthesized in vitro is then translocated directly into microsomes or the ER of permeabilized **yeast** cells. Conversion of the 26-kDa ER form of pro-alpha-factor to the high molecular weight Golgi form is dependent on the presence of ATP and soluble and membrane-bound factors. Differential centrifugation and fractionation on a sucrose gradient have shown that the ER and Golgi forms of alpha-factor are enriched in separate compartments after the transport reaction. These and other findings (see Ruohola et al., 1988, for a more complete discussion) indicate that conversion to the high molecular weight form of alpha-factor is the result of authentic intercompartmental transport. Permeabilized mammalian cells have been used to reconstitute transport from the ER to the Golgi complex. In these systems (Becker et al., 1987; Simons and Virta, 1987), a viral membrane glycoprotein protein (vesicular stomatitis virus **G protein**) is used as the marker protein. This protein is radiolabeled with [35S]methionine during virus infection, either before or after the cells are permeabilized. Radiolabeled **G protein**, residing in the ER, is then transported to the Golgi complex in the presence of an ATP-regenerating system. In the mammalian system the donor and acceptor compartments are retained within the permeabilized cells (Simons and Virta, 1987); however, on occasion the addition of an exogenous acceptor compartment is required (Beckers et al., 1987). The assay we developed (Ruohola et al., 1988) differs from the mammalian assay (Beckers et al., 1987) in that we introduce radiolabeled marker protein into the ER in vitro during translocation rather than during virus infection. In addition, in our assay the acceptor Golgi compartment is always provided exogenously to the

permeabilized cells. Therefore, if acceptor membranes are present in the PYC, they are not u[redacted]ized. Because the permeabilize[redacted]ells and the S3 fraction are prepared differently, the conditions used to prepare the cells may lead to inactivation or loss of the acceptor compartment. The in vitro assay will enable us to purify components involved in transporting proteins from the lumen of the ER to the Golgi complex. Antibody prepared to purified components can be used to clone the genes that code for these proteins.(ABSTRACT TRUNCATED AT 400 WORDS)

L1 ANSWER 23 OF 42 MEDLINE
AN 88223369 MEDLINE
DN 88223369
TI A GTP-binding protein required for **secretion** rapidly associates with **secretory** vesicles and the plasma membrane in **yeast**.
AU Goud B; Salminen A; Walworth N C; Novick P J
CS Department of Cell Biology, Yale University School of Medicine, New Haven, Connecticut 06510.
NC GM35370 (NIGMS)
GM07223 (NIGMS)
SO CELL, (1988 Jun 3) 53 (5) 753-68.
Journal code: CQ4. ISSN: 0092-8674.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 198809
AB SEC4, one of the 10 genes involved in the final stage of the **yeast** **secretory** pathway, encodes a ras-like, GTP-binding protein. In wild-type cells, Sec4 protein is located on the cytoplasmic face of both the plasma membrane and the **secretory** vesicles in transit to the cell surface. In all post-Golgi blocked sec mutants, Sec4p is predominantly associated with the **secretory** vesicles that accumulate as a result of the **secretory** block. Sec4p is synthesized as a soluble protein that rapidly (t1/2 less than or equal to 1 min) and tightly associates with **secretory** vesicles and the plasma membrane by virtue of a conformational change of a covalent modification. These data suggest that Sec4p may function as a "**G** " **protein** on the vesicle surface to transduce an intracellular signal needed to regulate transport between the Golgi apparatus and the plasma membrane.

L1 ANSWER 24 OF 42 MEDLINE
AN 87293904 MEDLINE
DN 87293904
TI Expression and glycosylation of the respiratory syncytial virus **G** **protein** in Saccharomyces cerevisiae.
AU Ding M X; Wen D Z; Schlesinger M J; Wertz G W; Ball L A
SO VIROLOGY, (1987 Aug) 159 (2) 450-3.
Journal code: XEA. ISSN: 0042-6822.
CY United States
DT Journal; Article; (JOURNAL ARTICLE)
LA English
FS Priority Journals; Cancer Journals
EM 198711
AB A cDNA encoding the entire amino acid sequence of the G glycoprotein of respiratory syncytial virus (RSV) was inserted into a **yeast** -Escherichia coli shuttle vector such that expression of the virus gene was regulated by the **yeast** GAL1 promoter. Transformation of Saccharomyces cerevisiae with the vector led to the formation of the **G protein** when cells were grown in the presence of galactose. Under these conditions the RSV G appeared as a 60- to 65-kDa glycosylated protein. Expression of the G cDNA in **secretory** mutants of S. cerevisiae yielded a protein of 35 kDa in a mutant unable to

glycosylate **secrete** proteins and a 65-kDa polypepti in a mutant unable to t sport proteins beyond the endop smic reticulum. The RSV protein formed in the latter mutant was converted to a 60-kDa protein by endoglycosidase H. Our results show that **yeast** can recognize the internal signal sequence of RSV **G protein** and add glycosyl groups to the polypeptide in the endoplasmic reticulum. Evidence is presented for both N- and O-linked glycosylation of the virus glycoprotein.

L1 ANSWER 25 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1999:444529 BIOSIS
DN PREV199900444529
TI Multiple sex pheromones and receptors of a mushroom-producing fungus elicit mating in **yeast**.
AU Fowler, Thomas J.; DeSimone, Susan M.; Mitton, Michael F.; Kurjan, Janet; Raper, Carlene A. (1)
CS (1) Department of Microbiology and Molecular Genetics, University of Vermont, Burlington, VT, 05405 USA
SO Molecular Biology of the Cell, (Aug., 1999) Vol. 10, No. 8, pp. 2559-2572.
ISSN: 1059-1524.
DT Article
LA English
SL English
AB The mushroom-producing fungus Schizophyllum commune has thousands of mating types defined, in part, by numerous lipopeptide pheromones and their **G protein**-linked receptors. Compatible combinations of pheromones and receptors encoded by different mating types regulate a pathway of sexual development leading to mushroom formation and meiosis. A complex set of pheromone-receptor interactions maximizes the likelihood of outbreeding; for example, a single pheromone can activate more than one receptor and a single receptor can be activated by more than one pheromone. The current study demonstrates that the sex pheromones and receptors of Schizophyllum, when expressed in Saccharomyces cerevisiae, can substitute for endogenous pheromone and receptor and induce the **yeast** pheromone response pathway through the **yeast** **G protein**. **Secretion** of active Schizophyllum pheromone requires some, but not all, of the biosynthetic machinery used by the **yeast** lipopeptide pheromone a-factor. The specificity of interaction among pheromone-receptor pairs in Schizophyllum was reproduced in **yeast**, thus providing a powerful system for exploring molecular aspects of pheromone-receptor interactions for a class of seven-transmembrane-domain receptors common to a wide range of organisms.

L1 ANSWER 26 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1999:16207 BIOSIS
DN PREV199900016207
TI Localization of the **G-protein** beta-subunit in **yeast** buds and mating projections.
AU Paiement, J. (1); Leeuw, T.; Roy, L.; Thomas, D.; Leberer, E; Whiteway, M.
CS (1) Dep. Path. Biol. Cell., Univ. Montreal, Montreal, PQ Canada
SO Molecular Biology of the Cell, (Nov., 1998) Vol. 9, No. SUPPL., pp. 362A.
Meeting Info.: 38th Annual Meeting of the American Society for Cell Biology San Francisco, California, USA December 12-16, 1998 American Society for Cell Biology
. ISSN: 1059-1524.
DT Conference
LA English

L1 ANSWER 27 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS

AN 1998:175308 BIOSIS
DN PREV199800175308
TI Structure of the Sec7 domain of the Arf exchange factor ARNO.
AU Cherfils, Jacqueline (1); Menetrey, Julie; Mathieu, Magali; Le Bras, Gerard; Robineau, Sylviane; Beraud-Dufour, Sophie; Antonny, Bruno; Chardin, Pierre
CS (1) Lab. Enzymol. Biochim. Structurales, CNRS UPR 9063, 91198 Gif-sur-Yvette France
SO Nature (London), (March 5, 1998) Vol. 392, No. 6671, pp. 101-105. ISSN: 0028-0836.
DT Article
LA English
AB Small G proteins switch from a resting, GDP-bound state to an active, GTP-bound state. As spontaneous GDP release is slow, guanine-nucleotide-exchange factors (GEFs) are required to promote fast activation of small G proteins through replacement of GDP with GTP in vivo. Families of GEFs with no sequence similarity to other GEF families have now been assigned to most families of small G proteins. In the case of the small **G protein** Arf1, the exchange of bound GDP for GTP promotes the coating of **secretory** vesicles in Golgi traffic. An exchange factor for human Arf1, ARNO, and two closely related proteins, named cytohesin 1 (ref. 4) and GPS1 (ref. 5), have been identified. These three proteins are modular proteins with an amino-terminal coiled-coil, a central Sec7-like domain and a carboxy-terminal pleckstrin homology domain. The Sec7 domain contains the exchange-factor activity. It was first found in Sec7, a **yeast** protein involved in **secretion**, and is present in several other proteins, including the **yeast** exchange factors for Arf, Gea1 and Gea2 (refs 7-9). Here we report the crystal structure of the Sec7 domain of human ARNO at 2 ANG resolution and the identification of the site of interaction of ARNO with Arf.

L1 ANSWER 28 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1997:500826 BIOSIS
DN PREV199799800029
TI A genetic selection for isolating cDNAs encoding **secreted** proteins.
AU Jacobs, Kenneth A. (1); Collins-Racie, Lisa A.; Colbert, Maureen; Duckett, McKeough; Golden-Fleet, Margaret; Kelleher, Kerry; Kriz, Ronald; La Vallie, Edward R.; Merberg, David; Spaulding, Vikki; Stover, Jen; Williamson, Mark J.; McCoy, John M.
CS (1) Genetics Inst. Inc., 87 Cambridge Park Drive, Cambridge, MA 02140 USA
SO Gene (Amsterdam), (1997) Vol. 198, No. 1-2, pp. 289-296. ISSN: 0378-1119.
DT Article
LA English
AB We describe a simple, rapid technique for simultaneously isolating large numbers of cDNAs encoding **secreted** proteins. The technique makes use of a facile genetic selection performed in a strain of Saccharomyces cerevisiae deleted for its endogenous invertase gene. A cDNA cloning vector which carries a modified invertase gene lacking its leader sequence is used in conjunction with this strain. Heterologous **secreted** genes fused appropriately upstream of this defective invertase provide the necessary signals to restore **secretion**, allowing the **yeast** to grow on sugars such as sucrose or raffinose. This microbial growth selection facilitates scanning cDNA libraries containing millions of clones, enabling the wholesale identification of novel **secreted** proteins without the need for specific bioassays. The technique is similar to one previously described (Klein et al. (1996) Proc. Natl. Acad. Sci. USA 93, 7108-7113). We describe results using a cDNA library derived from activated human peripheral blood mononuclear cells (PBMC). Genes identified from this library encoded signal sequences

of proteins of diverse structure, function, and cellular location such as cytokines, type 1 and type 2 transmembrane proteins, and proteins found in intracellular organelles. In addition, a number of novel **secreted** proteins were identified, including a chemokine and a novel **G-protein**-coupled receptor. Since signal sequences possess features conserved throughout evolution, the procedure can be used to isolate genes encoding **secreted** proteins from both eukaryotes and prokaryotes.

L1 ANSWER 29 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1997:320222 BIOSIS
DN PREV199799610710
TI Functional expression of bovine opsin in the methylotrophic **yeast** Pichia pastoris.
AU Abdulaev, Najmoutin G.; Popp, Michael P.; Smith, W. Clay; Ridge, Kevin D. (1)
CS (1) Cent. Advanced Res. Biotechnol., Natl. Inst. Standards Technol. Univ. Maryland Biotechnol. Inst., 9600 Gudelsky Drive, Rockville, MD 20850 USA
SO Protein Expression and Purification, (1997) Vol. 10, No. 1, pp. 61-69. ISSN: 1046-5928.
DT Article
LA English
AB The methylotrophic **yeast** Pichia pastoris was examined for functional expression of bovine opsin. An expression plasmid was constructed where the bovine opsin gene was placed downstream from the P. pastoris alcohol oxidase 1 gene promoter and fused at its amino-terminus to the acid phosphatase **secretion** signal. Quantitative-competitive PCR analysis of a stable **yeast** transformant showed that one copy of the opsin gene was integrated into the **yeast** genome. The expression level in this transformant corresponded to apprx 0.3 mg of opsin per liter of cell culture (A-600 = 1.0). Sucrose density sedimentation analysis indicated that the opsin was associated exclusively with the membrane fraction. Similar to retinal opsin, P. pastoris-expressed opsin migrated as a single band of apprx 37 kDa on SDS-PAGE and showed high mannose N-glycosylation. A portion of the expressed opsin (apprx 4-15%) reacted with 11-cis-retinal to form the rhodopsin chromophore (lambda-max 500 nm), and after purification showed ground and excited state spectral characteristics indistinguishable from those of the native pigment. Further, the metarhodopsin-11-mediated **G-protein**-activating potential of **yeast** expressed rhodopsin was similar to that of native rhodopsin. These results show that P. pastoris cells have the capacity to functionally express bovine opsin.

L1 ANSWER 30 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1997:108790 BIOSIS
DN PREV199799407993
TI Investigation of growth hormone releasing hormone receptor structure and activity using **yeast** expression technologies.
AU Kajkowski, Eileen M.; Price, Laura A.; Pausch, Mark H.; Young, Kathleen H.; Ozenberger, Bradley A. (1)
CS (1) American Cyanamid Co., P.O. Box 400, Princeton, NY 08543 USA
SO Journal of Receptor and Signal Transduction Research, (1997) Vol. 17, No. 1-3, pp. 293-303. ISSN: 1079-9893.
DT Article
LA English
AB Growth hormone releasing hormone (GHRH) is the positive regulator of growth hormone synthesis and **secretion** in the anterior pituitary. The peptide confers activity by binding to a seven transmembrane domain **G protein**-coupled receptor. Signal transduction proceeds through subsequent G-alpha-s stimulation of adenylyl cyclase. To investigate ligand/receptor and receptor/**G**

protein association the human GHRH receptor was expressed in a modified S. cerevisiae strain which allows for facile measurement of receptor activity by cell prototrophy mediated by a reporter gene coupled to the **yeast** pheromone response pith-way. GHRH-dependent signal activation in this system required the substitution of **yeast** G-alpha protein with proteins containing C-terminal regions of G-alpha-s. A D60G variant (analogous to the little mouse mutation) of the receptor failed to respond to agonist. In parallel studies, GHRH-29 and the N-terminal extracellular region of the receptor were expressed as Gal4 fusion proteins in a 2-hybrid assay. A specific interaction between these proteins was readily observed. The D60G mutation was engineered into the receptor fusion protein. This protein failed to interact with the ligand fusion, confirming the specificity of the association between unmodified proteins. These two **yeast** expression technologies should prove invaluable in additional structure/activity analyses of this ligand/receptor pair as well as other peptide ligands and receptors.

L1 ANSWER 31 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1997:15817 BIOSIS
DN PREV199799315020
TI A human exchange factor for ARF contains Sec7- and pleckstrin-homology domains.
AU Chardin, Pierre (1); Paris, Sonia; Antonny, Bruno; Robineau, Sylviane; Beraud-Dufour, Sophie; Jackson, Catherine L.; Chabre, Marc
CS (1) Inst. Pharmacol. Mol. Cell. CNRS, 660 route des Lucioles, 06560 Valbonne France
SO Nature (London), (1996) Vol. 384, No. 6608, pp. 481-484.
ISSN: 0028-0836.
DT Article
LA English
AB The small **G protein** ARF1 is involved in the coating of vesicles that bud from the Golgi compartments. Its activation is controlled by as-yet unidentified guanine-nucleotide exchange factors. Gea1, the first ARF exchange factor to be discovered in **yeast**, is a large protein containing a domain of homology with Sec7, another **yeast** protein that is also involved in **secretion**. Here we characterized a smaller human protein (relative molecular mass 47K) named ARNO, which contains a central Sec7 domain that promotes guanine-nucleotide exchange on ARF1. ARNO also contains an amino-terminal coiled-coil motif and a carboxy-terminal pleckstrin-homology (PH) domain. The PH domain mediates an enhancement of ARNO exchange activity by negatively charged phospholipid vesicles supplemented with phosphatidylinositol bisphosphate. The exchange activity of ARNO is not inhibited by brefeldin A, an agent known to block vesicular transport and inhibit the exchange activity on ARF1 in cell extracts. This suggests that a regulatory component which is sensitive to brefeldin A associates with ARNO in vivo, possibly through the amino-terminal coiled-coil. We propose that other proteins with a Sec7 domain regulate different members of the ARF family.

L1 ANSWER 32 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1996:519355 BIOSIS
DN PREV199699241711
TI The role of charged residues in determining transmembrane protein insertion orientation in **yeast**.
AU Harley, Carol A.; Tipper, Donald J.
CS Dep. Mol. Genetics Microbiol., Univ. Mass. Med. Sch., Worcester, MA 01655 USA
SO Journal of Biological Chemistry, (1996) Vol. 271, No. 40, pp. 24625-24633.
ISSN: 0021-9258.
DT Article
LA English
AB The first 79 residues of the **yeast** Ste2p **G protein**-coupled pheromone receptor, including the negatively

charged N-terminal domain, the first transmembrane segment, and the following positively charged cytoplasmic loop, has been fused to a Kex2p-cleavable beta-lactamase reporter. Insertion orientation was determined by analysis of cell-associated and **secreted** beta-lactamase activities and independently corroborated by analysis of membrane association and glycosylation patterns. This fusion inserts with exclusively N terminus exofacial (N-exo) topology, serving as a model type III membrane protein. Orientation is unaffected by removal of all three positively charged residues in the cytoplasmic loop or by deletion of all but 12 residues from the N-terminal domain. The residual -2 N-terminal charge apparently provides a signal sufficient to determine N-exo topology. This is entirely consistent with the statistically derived rule in which the charge difference, DELTA(C-N), counted for the 15 immediately flanking residues, is the primary topology determinant. Mutations altering DELTA(C-N) to zero favors N-exo insertion by 3 to 1, whereas increasingly negative values cause increasing inversion of orientation. All results are consistent with the charge difference rule and indicate that whereas positive charges promote cytoplasmic retention, negative charges promote translocation.

L1 ANSWER 33 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1996:462499 BIOSIS
DN PREV199699184855
TI **Yeast** alpha mating factor structure-activity relationship derived from genetically selected peptide agonists and antagonists of Ste2p.
AU Manfredi, John P. (1); Klein, Christine; Herrero, Juan J.; Byrd, Devon A.; Trueheart, Joshua; Wiesler, William T.; Fowlkes, Dana M.; Broach, James R.
CS (1) Cadus Pharm. Corp., 777 Old Saw Mill River Rd., Tarrytown, NY 10591-6705 USA
SO Molecular and Cellular Biology, (1996) Vol. 16, No. 9, pp. 4700-4709. ISSN: 0270-7306.
DT Article
LA English
AB alpha-Factor, a 13-amino-acid pheromone **secreted** by haploid alpha cells of Saccharomyces cerevisiae, binds to Ste2p, a seven-transmembrane, **G-protein**-coupled receptor present on haploid a cells, to activate a signal transduction pathway required for conjugation and mating. To determine the structural requirements for alpha-factor activity, we developed a genetic screen to identify from random and semirandom libraries novel peptides that function as agonists or antagonists of Ste2p. The selection scheme was based on autocrine strains constructed to **secrete** random peptides and respond by growth to those that were either agonists or antagonists of Ste2p. Analysis of a number of peptides obtained by this selection procedure indicates that Trp1, Trp3, Pro8, and Gly9 are important for agonist activity specifically. His2, Leu4, Leu6, Pro10, a hydrophobic residue 12, and an aromatic residue 13 are important for both agonist and antagonist activity. Our results also show that activation of Ste2p can be achieved with novel, unanticipated combinations of amino acids. Finally, the results suggest the utility of this selection scheme for identifying novel ligands for mammalian **G-protein**-coupled receptors heterologously expressed in S. cerevisiae.

L1 ANSWER 34 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1996:458940 BIOSIS
DN PREV199699181296
TI Regulation of membrane and subunit interactions by N-myristoylation of a

G protein alpha subunit in **yeast**.
AU Song, Jianping; Hirschman, Jodi; Gunn, Karen; Dohlman, Henrik G. (1)
CS (1) Boyer Cent. Mol. Med., Yale Univ. Sch. Med., 295 Congress Ave., New Haven, CT 06536-0812 USA
SO Journal of Biological Chemistry, (1996) Vol. 271, No. 34, pp. 20273-20283.
ISSN: 0021-9258.
DT Article
LA English
AB Initiation of the mating process in **yeast** Saccharomyces cerevisiae requires the action of **secreted** pheromones and **G protein**-coupled receptors. As in other eukaryotes, the **yeast G protein** alpha subunit undergoes N-myristoylation (GPA1 gene product, Gpa1p). This modification appears to be essential for function, since a myristoylation site mutation exhibits the null phenotype in vivo (gpa1-G2A). Here we examine how myristoylation affects Gpa1p activity in vitro. We show that the G2A mutant of Gpa1p, when fused with glutathione S-transferase, can still form a complex with the **G protein** beta-gamma subunits. The complex is stabilized by GDP and is dissociated upon treatment with guanosine 5'-O-(thiotriphosphate). In addition, there is no apparent difference in the relative binding affinity of G-beta-gamma for mutant and wild-type Gpa1p. Using sucrose density gradient fractionation of cell membranes, Gpa1p associates normally with the plasma membrane whereas Gpa1p-G2A is mislocalized to a microsomal membrane fraction. A portion of G-beta-gamma is also mislocalized in these cells, as it is in a gpa1-DELTA strain. In contrast, wild-type Gpa1p reaches the plasma membrane in cells that do not express G-beta-gamma or cell surface receptors. These findings indicate that mislocalization of Gpa1p-G2A is not caused by a redistribution of G-beta-gamma, nor is it the result of any difference in G-beta-gamma binding affinity. These data suggest that myristoylaiion is required for specific targeting of Gpa1p to the plasma membrane, where it is needed to interact with the receptor and to regulate the release of G-beta-gamma.

L1 ANSWER 35 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1996:123913 BIOSIS
DN PREV199698696048
TI Immunodominant epitopes defined by a **yeast**-expressed library of random fragments of the rabies virus glycoprotein map outside major antigenic sites.
AU Lafay, Florence (1); Benmansour, Abdenour; Chebli, Karim; Flamand, Anne
CS (1) Lab. Genetique Virus, CNRS, Avenue de la Terrasse, 91198 Gif-sur-Yvette Cedex France
SO Journal of General Virology, (1996) Vol. 77, No. 2, pp. 339-346.
ISSN: 0022-1317.
DT Article
LA English
AB Nineteen **yeast** colonies **secreting** rabies virus glycoprotein (G) peptides immunoreactive with polyclonal anti-rabies virus sera were selected from a random expression library. The peptides, around 80 amino acids long, spanned amino acids 54-494 of the **G protein**. These peptides, together with two constructions including, respectively, immunodominant sites II and III, were analysed for their immunoreactivity with 40 anti-**G protein** monoclonal antibodies (MAbs) composed of 12 MAbs that reacted with SDS-treated protein in Western blot under reducing conditions (WB+) and 28 representative MAbs that did not react after denaturation (WB-). This last category represents 98 % of anti-rabies virus G MAbs. None of the WB- MAbs bound peptides. Of the 12 WB+ MAbs, one bound two peptides situated before the transmembrane domain of the protein and six bound peptides overlapping

a region situated between amino acids 223 and 276. These six MAbs define a new antigenic region that would be considered 'immunodominant' if the peptide strategy had been used to study the antigenicity of the protein; however, this region is only recognized by about 1% of our MAbs. Three of these WB+ MAbs had significant neutralizing activity; two were used for the selection of antigenic mutants the region delimited by the peptides, confirming the (MAR mutants). Some mutants had a substitution within pertinence of both the peptide and escape mutant approaches. However, a few mutants had a substitution outside the peptide-delimited region, suggesting that remote mutations) could affect epitope accessibility in the native protein.

L1 ANSWER 36 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1995:528173 BIOSIS
DN PREV199598542473
TI Interaction of the protein nucleobindin with G-ai2, as revealed by the **yeast** two-hybrid system.
AU Mochizuki, Naoki; Hibi, Masahiko; Kanai, Yoshiyuki; Insel, Paul A. (1)
CS (1) Dep. Pharmacol., Univ. California San Diego, 9500 Gilman Drive, La Jolla, CA 92093-0636 USA
SO FEBS Letters, (1995) Vol. 373, No. 2, pp. 155-158.
ISSN: 0014-5793.
DT Article
LA English
AB The heterotrimeric **G protein**, G-alpha-i2, transduces signals from seven membrane spanning receptors to effectors such as adenylyl cyclase and ion channels. The purpose of this study was to identify these or other cellular proteins that interact with G-alpha-i2 by use of the **yeast** two-hybrid system. A human B cell cDNA library was screened by this system using full length G-alpha-i2. Four positive colonies were obtained. Two of the four were identified as nucleobindin, a calcium binding protein and a putative antigen to which anti-nuclear antibodies are generated in mice with a disorder that resembles systemic lupus erythematosus. Nucleobindin has a leucine zipper, EF hands, and a signal peptide sequence and is thought to localize to the nucleus as well as being **secreted**. The specificity of interaction between G-alpha-i2 and nucleobindin was confirmed by an in vitro binding assay using recombinant proteins. Transfection of G-alpha-i2 and nucleobindin in COS cells increased G-alpha-i2 expression relative to cells transfected with G-alpha-i2 and mock vector. Our results indicate that the **yeast** two-hybrid system provides a means to identify novel proteins that interact with G-alpha proteins. Nucleobindin appears to represent one of those proteins.

L1 ANSWER 37 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1995:201958 BIOSIS
DN PREV199598216258
TI **Yeast** as a model system for mammalian seven-transmembrane segment receptors.
AU Jeansonne, Neil E.
CS Dep. Pharmacol., East Carolina Univ. Med. Sch., Greenville, NC 27858 USA
SO Proceedings of the Society for Experimental Biology and Medicine, (1994) Vol. 206, No. 1, pp. 35-44.
ISSN: 0037-9727.
DT General Review
LA English
AB Investigators have used the budding **yeast** Saccharomyces cerevisiae as a model system in which to study the beta-adrenergic receptor, the T-cell receptor pathway, initiation of mammalian DNA replication, initiation of mammalian transcription, **secretion**, the CDC2 kinase system, cell cycle control, and aging, as well as the function of oncogenes. This list continues to grow with the discovery of

an immunoglobulin h[redacted]vy-chain binding homologue in y[redacted]st, an Rb binding protein ho[redacted]ogue, and a possible **yeast** arre[redacted]n. **Yeast** is relatively easy to maintain, to grow, and to genetically manipulate. A single gene can be overexpressed, selectively mutated or deleted from its chromosomal location. In this way, the in vivo function of a gene can be studied. It has become reasonable to consider **yeast** as a model system for studying the seven transmembrane segments (7-TMS) receptor family. Currently, subtypes of the beta-adrenergic receptor are being studied in **yeast**. The receptor and its G-alpha-**G-protein**, trigger the mating pheromone receptor pathway. This provides a powerful assay for determining receptor function. Studies expressing the muscarinic cholinergic receptor in **yeast** are underway. The **yeast** pheromone receptor belongs to this receptor family, sharing sequence and secondary structure homology. An effective strategy has been to identify a **yeast** pathway or process which is homologous to a mammalian system. The pathway is delineated in **yeast**, identifying other genetic components. Then **yeast** genes are used to screen for human homologues of these components. The putative human homologues are then expressed in **yeast** and in mammalian cells to determine function. When this type of "mixing and matching" works, **yeast** genetics can be a powerful tool.

L1 ANSWER 38 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1994:109864 BIOSIS
DN PREV199497122864
TI **Yeast** ts **secretory** mutation rgs1 is suppressed by the SEC4 gene of Saccharomyces cerevisiae.
AU Gerassimenko, Oxana G.
CS Centre Genetique Moleculaire, F-91198 Gif-sur-Ivette France
SO Current Genetics, (1994) Vol. 25, No. 2, pp. 178-179. ISSN: 0172-8083.
DT Article
LA English
AB **Yeast** rgs1 cells accumulate **secretory** vesicles in the cytoplasm and stop the **secretion** of proteins at the restrictive temperature. The ts mutation rgs1 may be suppressed by several different genes; the S. cerevisiae SEC4 gene, encoding the small **G-protein** involved in the late **secretory** stage, is one of them. Synthetic lethality of the double rgs1 sec4 mutant is demonstrated.

L1 ANSWER 39 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1988:458712 BIOSIS
DN BA86:100431
TI A GTP-BINDING PROTEIN REQUIRED FOR **SECRETION** RAPIDLY ASSOCIATES WITH **SECRETORY** VESICLES AND THE PLASMA MEMBRANE IN **YEAST** .
AU GOUD B; SALMINEN A; WALWORTH N C; NOVICK P J
CS DEP. CELL BIOL., YALE UNIV. SCH. MED., 333 CEDAR ST., P.O. BOX 3333, NEW HAVEN, CONN. 06510.
SO CELL, (1988) 53 (5), 753-768. CODEN: CELLB5. ISSN: 0092-8674.
FS BA; OLD
LA English
AB SEC4, one of the 10 genes involved in the final stage of the **yeast** **secretory** pathway, encodes a ras-like, GTP-binding protein. In wild-type cells, Sec4 protein is located on the cytoplasmic face of both the plasma membrane and the **secretory** vesicles in transit to the cell surface. In all post-Golgi block sec mutants, Sec4p is predominantly associated with the **secretory** vesicles that accumulate as a result of the **secretory** block. Sec4p is synthesized as a soluble protein that rapidly (t1/2 .ltoreq. 1 min) and tightly associates with **secretory** vesicles and the plasma membrane by virtue of a conformational change or a covalent modification. These data suggest that Sec4p may function as a "G" **protein** on the vesicle

surface to transdu n intracellular signal needed regulate transport between the Golgi ratus and the plasma membrane

L1 ANSWER 40 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1988:415479 BIOSIS
DN BA86:78091
TI A RAS-LIKE PROTEIN IS REQUIRED FOR A POST-GOLGI EVENT IN **YEAST SECRETION**.
AU SALMINEN A; NOVICK P J
CS DEP. CELL BIOL., YALE UNIV. SCH. MED., NEW HAVEN, CONN. 06510.
SO CELL, (1987) 49 (4), 527-538.
CODEN: CELLB5. ISSN: 0092-8674.
FS BA; OLD
LA English
AB **Secretion** is blocked at the post-Golgi stage within 5 min of a shift of sec4-8 cells from 25.degree.C to 37.degree.C. Analysis of SEC4 predicts a protein product of 23.5 kd molecular weight that shares 32% homology with ras proteins and is essential for growth. The regions of best homology are those involved in the binding and hydrolysis of GTP. Duplication of SEC4 suppresses postGolgi-blocked mutations in three sec genes. These mutations are lethal when combined with sec4-8 at 25.degree.C. Mutations that block elsewhere on the pathway are not suppressed by the SEC4 duplication and are not lethal when combined with sec4-8. We propose that the SEC4 product is a GTP-binding protein that plays an essential role in controlling a late stage of the **secretory** pathway.

L1 ANSWER 41 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1987:420687 BIOSIS
DN BA84:87349
TI EXPRESSION AND GLYCOSYLATION OF THE RESPIRATORY SYNCYTIAL VIRUS **G PROTEIN** IN SACCHAROMYCES-CEREVISIAE.
AU DING M; WEN D; SCHLESINGER M J; WERTZ G W; BALL L A
CS DEP. MICROBIOL. IMMUNOL., WASHINGTON UNIV. SCH. MED., ST. LOUIS, MO. 63110.
SO VIROLOGY, (1987) 159 (2), 450-453.
CODEN: VIRLAX. ISSN: 0042-6822.
FS BA; OLD
LA English
AB A cDNA encoding the entire amino acid sequence of the G glycoprotein of respiratory syncytial virus (RSV) was inserted into a **yeast** -Escherichia coli shuttle vector such that expression of the virus gene was regulated by the **yeast** GAL 1 promoter. Transformation of Saccharomyces cerevisiae with the vector led to the formation of the **G protein** when cells were grown in the presence of galactose. Under these conditions the RSV G appeared as a 60- to 65-kDa glycosylated protein. Expression of the G cDNA in **secretory** mutants of S. cerevisiae yielded a protein of 35 kDa in a mutant unable to
glycosylate **secreted** proteins and a 65-kDa polypeptide in a mutant unable to transport proteins beyond the endoplasmic reticulum. The RSV protein formed in the latter mutant was converted to a 60-kDa protein by endoglycosidase H. Our results show that **yeast** can recognize the internal signal sequence of RSV **G protein** and add glycosyl groups to the polypeptide in the endoplasmic reticulum. Evidence is presented for both N- and O-linked glycosylation of the virus glycoprotein.

L1 ANSWER 42 OF 42 BIOSIS COPYRIGHT 2000 BIOSIS
AN 1985:376031 BIOSIS
DN BA80:46023
TI DIGESTION ABSORPTION AND UTILIZATION OF SINGLE-CELL PROTEIN BY THE PRERUMINANT CALF ABOMASAL OUTFLOW AND ITS COMPOSITION FROM CALVES GIVEN MILK SUBSTITUTE DIETS CONTAINING VARYING AMOUNTS OF BACTERIAL OR **YEAST** PROTEIN.
AU SEDGMAN C A; ROY J H B; THOMAS J

CS NATL. INST. RES. D[redacted]YING, SHINFIELD, READING RG2 9
SO BR J NUTR, (1985) [redacted](3), 673-690.
CODEN: BJNUAV. ISSN: 0007-1145.
FS BA; OLD
LA English
AB Two experiments of Latin square design, with 4 Friesian bull calves fitted with re-entrant duodenal cannulas at 4-10 days of age, were made to study the effect of giving varying levels of single-cell protein on the abomasal outflow and composition of digesta. In expt (experiment) 1, diets in which 0, 220, 440 and 660 g/kg milk protein were replaced by the bacterial protein Pruteen were compared from 14 days of age. In expt 2, which began at about 61 days of age, a comparison was made of diets in which 0, 220, 440 and 660 g/kg milk protein were replaced by the **yeast** protein Toprina. Collection of abomasal outflow was made for 8 h after feeding for 2 days within each 6 day period of the Latin square design experiment. The amount of diet offered daily was 50 g dry matter/kg body-weight0.75. Polyethylene glycol (PEG), which was used as an indigestible marker, total nitrogen (TN), protein-N (PN), fat, and K, Na and Cl ion outflows were measured together with pepsin (EC 3.4.23.1) and chymosin (EC 3.4.23.4) activities, pH and titratable acidity. In expt 1 there was little difference in the outflow of liquid digesta between diets and about 0.9 of the dietary PEG was recovered within the 8 h collection period. With increasingg amounts of Pruteen in the diet, outflows of TN, PN, fat and Na+ increased, and the pH of digesta increased. However, the volume of 'apparent section' into the abomasum (outflow-intake), pepsin activity, chymosin activity, titratable acidity, (outflow of Cl--outflow of Na+) as a measure of outflow of HCl, and outflows of K+ and of Cl- were reduced. All outflows decreased with the time interval after feeding, except (Cl--Na+) outflow. In expt 2, the same trends as in expt 1 were apparent, but since 1 calf had to be slaughtered and the experiment had to be analyzed as a randomized block, only PN and K+ outflows and pH were significantly affected by dietary treatment, with K+ outflow increasing, rather than decreasing, with increasing concentration of single-cell protein in the diet. Reduced proteolysis in the abomasum, associated with a faster and greater outflow of protein as a result of poor or non coagulation of protein in the abomasum, and a reduction in **secretion** of enzymes and in acidity may partly explain the poor protein digestibility and growth rate obtained in other experiments when diets containing more than 100 g single-cell protein/kg diet (.apprx. 200 **g protein**/kg total protein) were given to young calves.

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ALL	l2 and library	229	L3
ALL	l1 and heterologous	243	L2
ALL	yeast and (g-protein or GPCR or GPCRs) and (secret$ or peptide library)	326	L1